TE: This evaluation kit contain components to perform 10 tests only

EXHIBIT A

VecTest™

West Nile Virus/ Saint Louis Encephalitis Antigen Panel Assay

Medical Analysis Systems, Inc.

Intended Use

The VecTest™ West Nile Virus (WNV) and Saint Louis Encephalitis (SLE) Antigen Panel Assay is a rapid immunochromatographic assay intended for the qualitative determination of WNV and SLE antigen in infected mosquitoes. Results from this assay can enable public health teams to:

- Continuously monitor mosquito vectors
- Focus vector control and eradication efforts
- Deliver cost-effective prevention of disease

Summary

Assays which detect the disease-causing agents and pathogens in field populations of arthropods, such as mosquitoes, make it possible to monitor the spread of the disease, to identify areas where there is risk of contracting disease, and to more efficiently target arthropod control measures. By monitoring infection rates and viral activity in nature, it may be possible to predict the threat of epidemic transmission in a population. While growth of the virus in cell culture or PCR-based molecular methods remain the standard for virus identification, the availability of a rapid, stable, simple, sensitive and specific diagnostic tool makes virus surveillance more expedient and cost-effective.

Monoclonal antibodies against SLE, WNV and the Flavivirus group have been employed in developing enzyme-linked immunosorbent assays (ELISA) that have been valuable tools in epidemiological studies and in assessing the risk and identification of vectors. Specific antibodies, extensively characterized by ELISA, have been employed in the development of the VecTest™ WNV/SLE Antigen Panel Assay. The use of the ELISA in monitoring infection rates in vector mosquitoes has been a great improvement over the methods of virus isolation in cell culture or in mice. However, the ELISA format is not always practical nor expedient enough to give the information required in medical threat assessments. The ELISA is a multi-component, 4-6 hour assay requiring specialized equipment, refrigeration of reagents, and highly trained personnel. Access to such facilities and equipment is usually unavailable in the field where the specimens are obtained and testing must wait until it can be done in a suitable laboratory environment.

The VecTest™ WNV/SLE Antigen Panel Assay is a rapid wicking assay that identifies the presence or absence of viral antigen specific to WNV or SLE in mosquitoes. The assay is a rapid, one step procedure using a wicking test strip. Rapid results, ambient storage and lack of specialized equipment needed in testing samples are the big advantages of the wicking WNV/SLE antigen assay over the ELISA, and prior training is not necessary.

Principle

The VecTest™ WNV/SLE Antigen Panel Assay is based on the dual monoclonal antibody "sandwich" principle. The test is initiated by placing one VecTest™ WNV/SLE dipstick into a 250 µl (0.25 ml) homogenized solution of up to ten mosquitoes. Antigen present in the solution binds to the specific antibody with a gold sol particle label. As the antigen-antibody-gold complexes migrate through the test zone containing immobilized WNV and SLE antibodies, they bind to the corresponding immobilized antibodies forming a "sandwich". The unbound dye complexes migrate out of the test zone and can be captured later in the control zone. A reddish-purple line develops on the specific area of the test zone when antigen is present. The control line in the control zone, farthest from the sample, should always develop provided the test has been carried out correctly.

Reagents

VecTest™ WNV/SLE Antigen Panel Assay is sold as a unit of 50 single-use dipsticks. Each VecTest™ WNV/SLE Antigen Panel Assay kit contains the following:

50 VecTest™ WNV/SLE Antigen Panel Assay dipsticks in a canister with a desiccant cap

Test Zone:
- Monoclonal antibodies to WNV and SLE immobilized on a membrane

Control Zone:
- Polyclonal goat antibody to mouse immunoglobulins immobilized on a membrane

Conjugate Pad:
- Gold complexed to monoclonal antibodies to flavivirus

Grinding Solution (125 ml)

St rage and Stability
The VecTest™ WNV/SLE Antigen Assay dipsticks and unopened Grinding Solution are stable up to the expiration date when stored at room temperature (10-30°C or 50-86°F).

To obtain good test results, dipsticks should be kept tightly closed in the provided container until ready for use.

Specimen Collection and Preparation

Mosquitoes: Use mosquitoes captured using the method(s) which best support(s) the sampling of encephalitis or West Nile fever vectors in the geographic area in which testing is to be done.

Storage: Mosquitoes should be used immediately or should be dried for later use. Homogenized samples of mosquitoes should be stored at -20°C until they can be processed.

Procedure

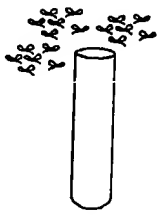
Materials Provided
- VecTest™ WNV Antigen Assay dipsticks (total of 50) in two canisters with desiccant cap
- 3 bottles of Grinding Solution (44 mL each)
- 50 culture tubes
- 200 copper coated BBs in a container
- 50 conical tubes
- 5 ten-hole tube racks
- Instructional insert

Materials Required (but not provided)
- Vortex machine
- Pipette
- Timing device
- Centrifuge (optional)
- Motorized grinder (optional)

Guidelines for Handling the Grinding Solution
Before use, the Grinding Solution should be mixed by gently inverting the bottle five times.

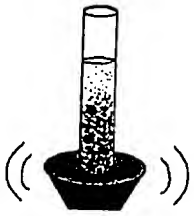
Procedure Outline

1. Place up to 50 female mosquitoes into a plastic culture tube provided in the kit.

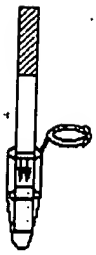


2. Dispense 2.5 mL of Grinding Solution onto the mosquitoes and add four copper-coated BBs provided in the kit.

3. Vortex the capped tube for 1 minute at high speed until the mosquito pool is homogenized into a slurry. (A centrifugation step may be performed to remove excess mosquito debris before running the test.)

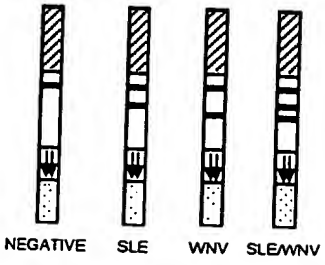


4. Dispense 250 µl of mosquito homogenate into a conical tube provided, place the tube into the tube stand provided, and insert a test strip from the canister with the arrows pointing down. (Replace the desiccant cap on the canister to protect the remaining strips from moisture.)



Wait 15 minutes for the test to be completed.

5. Determine the test results by removing the test strip and comparing it to the pictorial sample provided on the back of this insert.



On-board Control Principle
The control line develops as a result of an antibody-capture principle. The flow of the sample up the dipstick carries unreacted anti-flavivirus conjugated with gold sol particles. When the sample reaches the Control Zone, these unreacted conjugates bind to the antibody immobilized on the membrane and a signal is produced.

Quality Control Results
The test results are not valid if the control line does not develop. These results should be disregarded, the dipstick discarded, and the test run again. The test should only be interpreted as positive if two or three lines develop.

Results

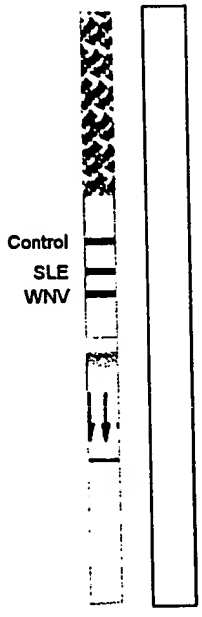
The presence of only a control line on the dipstick indicates a negative test result.

The presence of two or three lines indicates the presence of WNV and/or SLE antigen.

Results should be read within 30 minutes of performing the assay.

Test Strip Comparison

Place your strip in the box below for comparison of signal pattern(s).



Limitations

It is not recommended to test more than 10 mosquitoes per 250 µl (0.25 ml) of grinding solution with one VecTest™ WNV/SLE dipstick. The amount of mosquito debris may interfere with the interpretation of results. The volume of the homogenized solution should be 250 µl +/- 25 µl. The amount of foam remaining in the solution after motorized homogenation should be minimized.

The VecTest™ WNV/SLE dipstick detects the presence of WNV or SLE antigen in many vector species. Mosquitoes of the genus *Culex, Aedes* and many species within the genus, are known to be the most common carrier for West Nile Virus and Saint Louis Encephalitis, and can be tested with the dipstick.

Specific Performance Characteristics

Sensitivity/specificity studies were performed during assay development in a laboratory setting. The WNV assay sensitivity is 10^3 fold dilution of a culture antigen, and 5-6 Log10 PFU/ml WNV in laboratory infected mosquito pools when individual WNV assay was tested. The SLE assay sensitivity is 10^4 fold dilution of a recombinant antigen, and 3.5-4 Log10 PFU/ml SLE virus in laboratory infected mosquito pools when individual SLE assay was tested. No cross-reactivity was seen between the WNV and SLE antigens. One infected mosquito could be detected in a pool of 50 or 100 mosquitoes ground in a 2.5 ml sample according to results from CDC (Fort Collins,CO).

When mosquitoes infected with WEE and EEE, as well as recombinant WEE and EEE antigens diluted individually in grinding solution were tested, there was no cross-reactivity noted using the individual SLE assay.

The WNV/SLE panel assay was tested in CDC 1/2001 with laboratory infected mosquitoes to confirm data produced by the individual WNV or SLE assay. Quantitative results are pending from these new testings.

Correlation Data

Mosquito pools of (1) 1 positive and 49 negatives or (2) 50 negatives, ground in 1.25 ml of Grinding Solution, were prepared and tested with the different asays: VecTest™ SLE and WNV dipsticks, ELISA, and TaqMan RT-PCR.

Laboratory Results (May, 2000):

SLE assay

Mosquito Pool	VecTest™ SLE dipstick	ELISA OD at 450nm (Of antigen being tested)	TaqMan® RT-PCR (log10 PFU/ml)
SLE+ #1	+	>18.75	3.9
SLE+ #2	+	>18.75	4.0
SLE+ #3	+	>18.75	3.5
WEE+ #3	-	10.5	4.4
EEE+ #3	-	5.24	5.1
NEGATIVE	-	0	0

WNV assay

	TaqMan results (PFU/5ul) diluted 1:1000	Calculated PFU/mL	Log 10 calculated PFU/mL	Dipstick results
WNV 1	48300	9660000	7.0	3+
WNV 2	84000	16800000	7.2	3+
WNV 3	67900	13580000	7.1	3+

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